Journal Pre-proof

Robust and specific secretory IgA against SARS-CoV-2 detected in human milk

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1   **Robust and specific secretory IgA against SARS-CoV-2 detected in human milk**

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4

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16
17

18   **Summary**
19
20   The SARS-CoV-2 immune response in human milk has not yet been examined, though protecting

21   infants and young children from COVID-19 is critical for limiting community transmission, and

22   preventing serious illness and death. Here, milk samples from 8 COVID-19-recovered and 7 COVID-

23   19-suspected donors were tested for antibody (Ab) binding to the SARS-CoV-2 Spike protein. All

24   samples exhibited significant specific IgA reactivity to the full Spike, while 80% exhibited significant

25   IgA and secretory (s)Ab binding to the receptor binding domain (RBD). Additionally, 67% of samples

26   exhibited IgG and/or IgM binding to RBD. IgA and sAb titers were highly correlated, indicating most

27   IgA to be sIgA. Overall, these data indicate that a robust sIgA-dominant SARS-CoV-2 Ab response in

28   human milk after infection should be expected in a significant majority of individuals. Further research

29   is highly warranted to determine Ab functionality, and the potential for exploiting extracted milk sIgA

30   for therapeutic use.

31

Introduction

In the 7 months following the first reported case of coronavirus disease 2019 (COVID-19) in

December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over

21 million people and caused more than 750,000 deaths worldwide (World Health Organization,

2020). Though COVID-19 pathology in children is typically mild compared to adults, approximately

10% of infants under one year of age who contract the virus will experience severe COVID-19

illness requiring advanced care (CDC COVID-19 Response Team, 2020; Dong et al., 2020). Given

that COVID-19 pathology does not always correlate with transmissibility(Li et al., 2020; Wei et al.,

2020), resent studies suggest that infants and young children can transmit SARS-CoV-2 (CDC

COVID-19 Response Team, 2020; Lopez et al., 2020). As well, recently it has become evident that a

minority of children will experience a 'Multisystem Inflammatory Syndrome in Children (MIS-C)

associated with COVID-19' after SARS-CoV-2 infection, which has been fatal in certain cases

(Riphagen et al., 2020; Verdoni et al., 2020). For all of these reasons, protecting this population from

infection is essential. One potential protective mechanism might be passive immunity via

breastfeeding from a previously-infected mother or milk donor.

To date, almost nothing is known about the antibody (Ab) response in human milk to SARS-CoV-2

(Lackey et al., 2020). One preprint by Yu and colleagues (2020) reported that 2 milk samples produced

by a 32-year-old Chinese mother of a 13-month-old boy were positive for SARS-CoV-2 IgG and

negative for IgM on days 8 and 24 after hospital admission. Additional research is urgently needed to

test human milk for SARS-CoV-2 specific antibodies (Abs) and their functions. Knowing the typology

and degree of COVID-19 specific Abs in human milk will help inform smart policy and treatment

decisions for the many pregnant and breastfeeding mothers who are or will become infected by SARS-

55 CoV-2.

56

57 Certainly, any evidence of SARS-CoV-2 specific Abs in human milk must also be carefully weighed

58 against the risks of potential vertical transmission of SARS-CoV-2 through human milk (for a review,

59 see Centeno-Tablante et al., 2020). At the time of writing, 9 of the 68 milk samples obtained from

60 donors infected with SARS-CoV-2 that have been tested to date were found to contain SARS-CoV-2

61 RNA, although there is no evidence of SARS-CoV-2 transmission through breastfeeding and no

62 replication-competent virus has been found in any milk samples (Centeno-Tablante et al., 2020;

63 Chambers et al., 2020; Groß et al., 2020; Wu et al., 2020).

64

65 Despite the dearth of research, there are strong reasons to expect some SARS-CoV-2-specific Abs to be

66 present in the milk of previously infected mothers. Given that milk IgG originates predominantly from

67 serum, it follows that specific IgG in milk should appear contemporaneously with the previously

68 reported serum SARS-CoV-2 Ab response, though IgG comprises only ~2% of milk Ig(Hurley and

69 Theil, 2011). Approximately 90% of human milk Ab is IgA and ~8% IgM, nearly all in secretory (s)

70 form (sIgA/sIgM; polymeric Abs (Abs) complexed to j-chain and secretory component (SC) proteins

71 (Brandtzaeg, 2010; Demers-Mathieu et al., 2018; Hurley and Theil, 2011). These secretory Abs are

72 marked with SC as part of the mechanism by which they are secreted into the milk, whereby they are

73 actively transported via the polymeric immunoglobulin receptor (pIgR), from which SC is cleaved

74 (Brandtzaeg, 2010). SC is essential for protecting these Abs from relatively harsh mucosal environments

75 such as the infant mouth and gut. The majority of sIgA/sIgM derives from the gut-associated lymphoid

76 tissue (GALT), though there is also homing of B cells from other mucosa (i.e. the respiratory system) to

77 the mammary gland. Therefore, we predicted that SARS-CoV-2-specific sIgA/sIgM to be present in the

78 milk of previously infected mothers.

79

80 Here, we sought to characterize the types and magnitude of targeted Abs in human milk against SARS-

81 CoV-2. Specifically, this report details the findings regarding SARS-CoV-2-reactive IgA, IgG, IgM, and

82 total sAb in 15 milk samples obtained from donors previously-infected with COVID-19, 3-4 weeks after

83 symptoms had abated.

84

85 **Results**

86 **All human milk samples obtained form COVID-19-recovered donors contains significant levels of**

87 **SARS-CoV-2-specific IgA**.

88 Milk samples were initially evaluated for IgA binding reactivity by human IgA-specific ELISA to the

89 full trimeric SARS-CoV-2 Spike (Fig. 1). It was evident that all samples obtained from COVID-19-

90 recovered donors (100%), in undiluted form, exhibited binding activity significantly above that of the

91 pre-pandemic control milk samples, which did exhibit some low-level non-specific or cross-reactive

92 activity (Fig. 1a). Milk samples were titrated and endpoint titer values were determined. It was found

93 that all COVID-19-recovered samples exhibited endpoint titers significantly higher than control samples

94 (Fig. 1b).

95

96 **Most milk from COVID-19-recovered donors exhibits IgA and secretory Ab reactivity against the**

97 **receptor binding domain (RBD) of the SARS-CoV-2 Spike.** Samples were further tested separately

98 for Ab binding reactivity to the RBD of the Spike protein. Twelve of 15 samples (80%) obtained from

99 previously-COVID-19-infected donors exhibited significant IgA binding activity to RBD, as determined

100   by human IgA-specific ELISA, compared to controls in undiluted form (Fig. 2a). Milk samples were

101   titrated and endpoint titer values determined. It was found that 9 of the 12 samples with reactive IgA to

102   RBD when undiluted (75%) exhibited significant endpoint titers (Fig. 2c). Notably, all 12 of the milk

103   samples with significant IgA reactivity in undiluted form to RBD (100%) were also positive for RBD-

104   specific secretory Ab reactivity as determined by human SC-specific ELISA (Fig. 2b). All 12 milk

105   samples also exhibited positive secretory Ab endpoint titers upon dilution compared to controls (Fig.

106   2d).

107

108   **The RBD-specific IgA response in milk is dominant and not necessarily concurrent with a**

109   **measurable IgG or IgM response.** Individual profiles of Ab subclasses detected in the milk samples

110   from COVID-19-recovered mothers are reported in Table 1. Notably, specific IgG and IgM were only

111   measurable for a subset of samples, as determined by human IgG- and IgM-specific ELISAs,

112   respectively. Of the 12 milk samples shown to be positive for IgA reactivity, 8 were also positive for

113   IgG and/or IgM activity (67%). Four samples exhibited significant IgG and IgM reactivity to RBD

114   (COV107, COV112, COV113, COV117; Fig. 3). An additional 3 samples exhibited significant IgG

115   reactivity but not IgM (COV108b, COV111, COV116), and 1 sample exhibited IgM reactivity but not

116   IgG (COV101; Fig. 3).

117

118   **Milk from COVID-19-recovered donors exhibits significantly greater IgA, secretory Ab, and IgG**

119   **binding against RBD compared to controls.** Overall, OD values of undiluted milk obtained from

120   COVID-19-recovered donors and pre-pandemic controls for each assay were grouped and compared,

121   and it was found that the COVID-19-recovered group mean values were significantly greater for IgA

122   ($p<0.0001$), secretory Abs ($p<0.0001$), and IgG ($p=0.004$), but not for IgM (Fig. 4a-d). OD values for

123   undiluted milk were compared between each Ab subclass. The IgA and secretory Ab OD values for

124   undiluted milk were found to be highly correlated (r=0.81, p<0.0001; Fig. 4e). IgM and IgG OD values

125   were found to be modestly positively correlated (r=0.49, p=0.009; Fig. 4f). No other correlations were

126   found (data not shown).

127

128   **Discussion**

129   All milk samples obtained from COVID-19-recovered participants were positive for spike-reactive Ab

130   of at least one subclass, namely, IgA. Eighty percent of these samples were specifically reactive against

131   the RBD, with most (75%) exhibiting RBD binding activity that was quantitatively and/or qualitatively

132   high such that endpoint titers were significantly above the background activity of the pre-pandemic

133   controls. The samples analyzed represent only a snapshot of what is likely a dynamic immune response.

134   A much larger sample size and long-term follow-up study is needed to better understand the time-course

135   of SARS-CoV-2 immunity in milk, as well as whether a typical response is truly protective for breastfed

136   babies.

137

138   Though it might be expected that the milk Ab response would be reflective of systemic immunity (i.e.,

139   milk Ab should generally mirror serum Ab), only a small fraction of milk Ab originates from serum –

140   likely less than 10%, and only ~2% of milk Ab is IgG (Yu et al., 2020). Human milk Ab is ~90% IgA

141   and 8% IgM, nearly all sIgA/sIgM. The B cells that ultimately produce sIgA/sIgM originate mainly

142   from the GALT, known as the *entero-mammary link*, with some proportion originating from other

143   mucosa such as the respiratory system (Ahlstedt et al., 1977; Brandtzaeg, 2010; Kleinman and Walker,

144   1979). As such, there is much precedent for milk Ab composition and specificity being unique from that

145   found in blood. Though we did not compare the milk donors' blood Ab titers to the milk data, it was

146 evident that most of the samples contained SARS-CoV-2-reactive IgA without necessarily containing

147 measurable IgG and/or IgM, which particularly in the case of IgG, would likely be derived in large part

148 from the serum. Notably, IgG and IgM reactivities in undiluted milk exhibited a moderate correlation. It

149 may also be that as total IgG and IgM are so much lower in milk than IgA, that this ELISA lacked the

150 sensitivity to pick up very low-titer responses.

151

152 Though it has been determined by previous studies that most IgA in human milk is sIgA, our ELISA

153 could not determine with certainty that the IgA (or IgM) measured was of the secretory type or not

154 (Brandtzaeg, 2010). The assay measuring secretory Ab reactivity employs a secondary Ab specific for

155 the SC, which can be free, or bound to Ab. Notably, all samples exhibiting positive IgA reactivity also

156 exhibited positive SC reactivity, and a very strong positive correlation was present when comparing the

157 OD values of undiluted milk for the IgA and SC assays. This suggests that a very high proportion of the

158 SARS-CoV-2-reactive IgA measured herein was sIgA. This is extremely relevant to the possibility of

159 using extracted milk Ab as a COVID-19 therapy – for anyone with severe COVID-19 disease, as sIgA is

160 unique from the IgG-dominant convalescent plasma or purified plasma immunoglobulin being tested

161 currently (Bloch et al., 2020). Extracted milk sIgA used therapeutically would likely survive well upon

162 targeted respiratory administration, with a much lower dose of Ab likely needed for efficacy compared

163 to systemically-administered convalescent plasma or purified plasma immunoglobulin. Notably, the

164 purified material would need to be extensively safety-tested, including ensuring it as free of SARS-CoV-

165 2 material. Alternatively, recombinantly-produced, monoclonal or polyclonal Spike-specific sIgA could

166 be employed as a similar therapeutic.

167

168 As we continue with our comprehensive studies on the human milk immune response to SARS-CoV-2,

169 we aim to ultimately determine the efficacy of 'convalescent milk Ab' as a treatment for COVID-19,

170 and the utility of these Abs to prevent or mitigate infant SARS-CoV-2 infection. These data will have

171 implications beyond the pandemic, as they will serve to fill relatively large knowledge gaps regarding

172 human milk immunology.

173

174

175 **Limitations of study**

176 Not all samples analyzed herein were obtained from participants with PCR-confirmed SARS-CoV-2

177 infection, and a larger sample set of confirmed infections should be studied to confirm these findings.

178 The samples analyzed represent only a snapshot of what is likely a dynamic immune response. Long-

179 term follow-up is needed to understand the kinetics of this response. Further functional studies, in

180 particular neutralization data, is essential to understand this response.

181

182 **Resource Availability**

183 Further information and requests for resources and reagents should be directed to the lead contact,

184 Rebecca Powell: Rebecca.Powell@mssm.edu

185

186 **Materials Availability**

187 No unique reagents have been generated in this study. The SARS-CoV-2 antigens used in this study are

188 available from the Krammer Lab upon reasonable request, and similar antigens are available

189 commercially. All other reagents are commercially available.

190

191 **Data and Code Availability**

192 The data that support the findings of this study are available from the Lead Contact upon request.

193

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199

Authors and Contributors

201 Alisa Fox performed all experiments described in the paper, helped with data analysis, and gave final
202 approval of the final manuscript. Jessica Marino helped with data collection, gave feedback on drafts of
203 the paper, and gave final approval of the final manuscript. Fatima Amanat and Florian Krammer
204 developed and provided SARS-CoV-2 antigens and gave final approval of the final manuscript. Jennifer
205 Hahn-Holbrook helped with data collection, gave feedback on drafts of the paper, and gave final
206 approval of the final manuscript. Susan Zolla-Pazner gave feedback on drafts of the paper, and gave
207 final approval of the final manuscript. Rebecca L. Powell conceived of the research project, oversaw
208 data collection, preformed data analysis and interpretation, drafted and revised manuscript and gave
209 final approval of the manuscript.

210

Declaration of Interests

212 Mount Sinai has licensed serological assays to commercial entities and has filed for patent protection for

213 SARS-CoV-2 serological and milk assays.

214

215

Figure Legends

217   **Figure 1.** **All human milk samples collected from COVID-19-recovered donors exhibit IgA**

218   **reactivity against the full SARS-CoV-2 spike trimer**. (A) Full titration against Spike. NEG (i.e.

219   negative)/segmented lines: pre-pandemic controls. COV/solid lines: milk from COVID-19-recovered

220   donors. (B) Endpoint dilution titers. Experiments were performed in duplicate and repeated twice. Mean

221   with SEM is shown. Dotted lines indicate positive cutoff value (mean OD or endpoint titer of negative

222   control milk samples + 2*SD). Segmented line indicates 10x positive cutoff value.

223

224   **Figure 2**. **Eighty percent of human milk samples collected from COVID-19-recovered donors**

225   **exhibit IgA and secretory antibody reactivity against the receptor binding domain (RBD) of the**

226   **SARS-CoV-2 Spike**. (A) Full titration against RBD, measuring IgA binding. (B) IgA endpoint dilution

227   titers. (C) Full titration against RBD, measuring secretory antibody binding. (D) Secretory antibody

228   endpoint dilution titers. NEG (i.e. negative)/segmented lines: pre-pandemic controls. COV/solid lines:

229   milk from COVID-19-recovered donors. Experiments were performed in duplicate and repeated twice.

230   Mean with SEM is shown. Dotted lines indicate positive cutoff value (mean OD or endpoint titer of

231   negative control milk samples + 2*SD).

232

233   **Figure 3**. **The RBD-specific IgA response in milk is dominant and not necessarily concurrent with**

234   **a measurable IgG or IgM response.** Full titrations against RBD, measuring IgG (A) and IgM (B)

235   binding are shown. NEG (i.e. negative)/segmented lines: pre-pandemic controls. COV/solid lines: milk

236   from COVID-19-recovered donors. Experiments were performed in duplicate and repeated twice. Mean

237   with SEM is shown. Dotted lines indicate positive cutoff value (mean OD or endpoint titer of negative

238   control milk samples + 2*SD).

239

240 **Figure 4**. **Milk from COVID-19-recovered donors exhibits significantly greater IgA, secretory**

241 **antibody, and IgG binding against RBD compared to controls.** Grouped OD values for undiluted

242 milk are shown for IgA (A), secretory antibody (B), IgG (C), and IgM (D). Experiments were performed

243 in duplicate and repeated twice. Mean with SEM is shown. (E) Correlated IgA vs. secretory Ab binding

244 activity. (F) Correlated IgG vs. IgM binding activity. For correlation tests, OD values for undiluted milk

245 were used in 2-tailed Spearman correlation tests. SC: secretory component.

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326

Table 1. Summary Data

Sample ID	COVID-19 Confirmed or Suspected (C/S)	Months Post-partum	Infected ante- or post-partum (A/P)	Reactivity against SARS-CoV-2 Spike			
				IgA	SC†	IgM	IgG
COV101	C	4	P	+	+	+	-
COV102	C	1	A#	+*	+^	-	-
COV103c	C	4	P	+	+	-	-
COV104	S	23	P	+	+	-	-
COV105b	S	6	P	+*	+^	-	-
COV106c	S	8	P	+	+	-	-
COV107	S	32	P	+	+	+	+
COV108b	C	4	P	+*	+^	-	+
COV109b	S	3	P	+	+	-	-
COV110	S	14	P	+	+	-	-
COV111	S	7	P	+	+	-	+
COV112	C	1	A#	+	+	+	+
COV113	C	7	P	+	+	+	+
COV116	C	6	P	+	+	-	+
COV117	C	4	P	+	+	+	+

*sample was positive against Spike but negative against RBD

†Secretory component

^SC reactivity against spike is presumed based on RBD data, but was not tested

#Participants were infected within the last 6 weeks of pregnancy

A ELISA reactivity of IgA in human milk against the full SARS-CoV-2 spike trimer
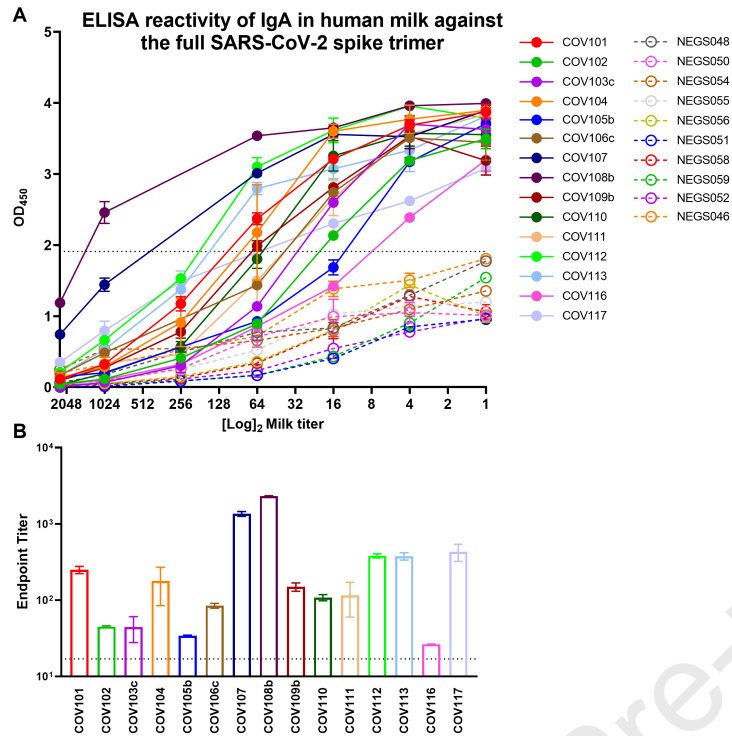
B

Figure 1. All human milk samples collected from COVID-19-recovered donors exhibit IgA reactivity against the full SARS-CoV-2 spike trimer.
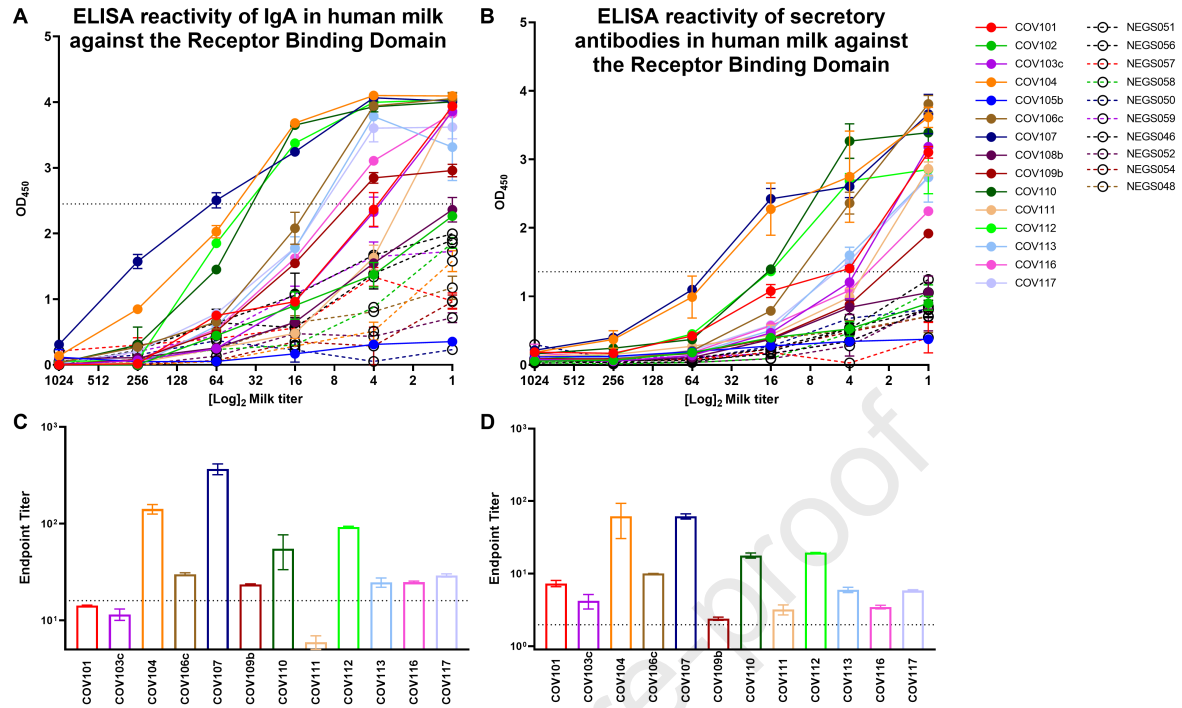
Figure 2. Eighty percent of human milk samples collected from COVID-19-recovered donors exhibit IgA and secretory antibody reactivity against the receptor binding domain (RBD) of the SARS-CoV-2 Spike.
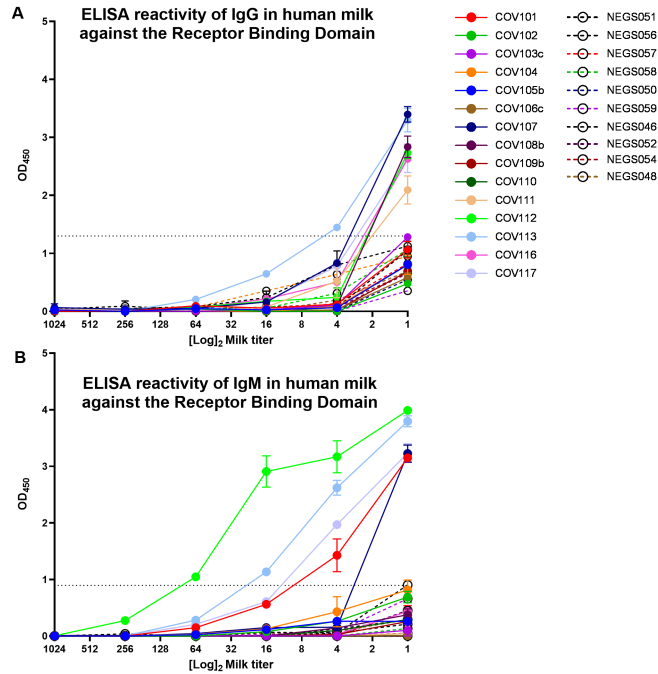
A ELISA reactivity of IgG in human milk against the Receptor Binding Domain

B ELISA reactivity of IgM in human milk against the Receptor Binding Domain

Figure 3. The RBD-specific IgA response in milk is dominant and not necessarily concurrent with a measurable IgG or IgM response.
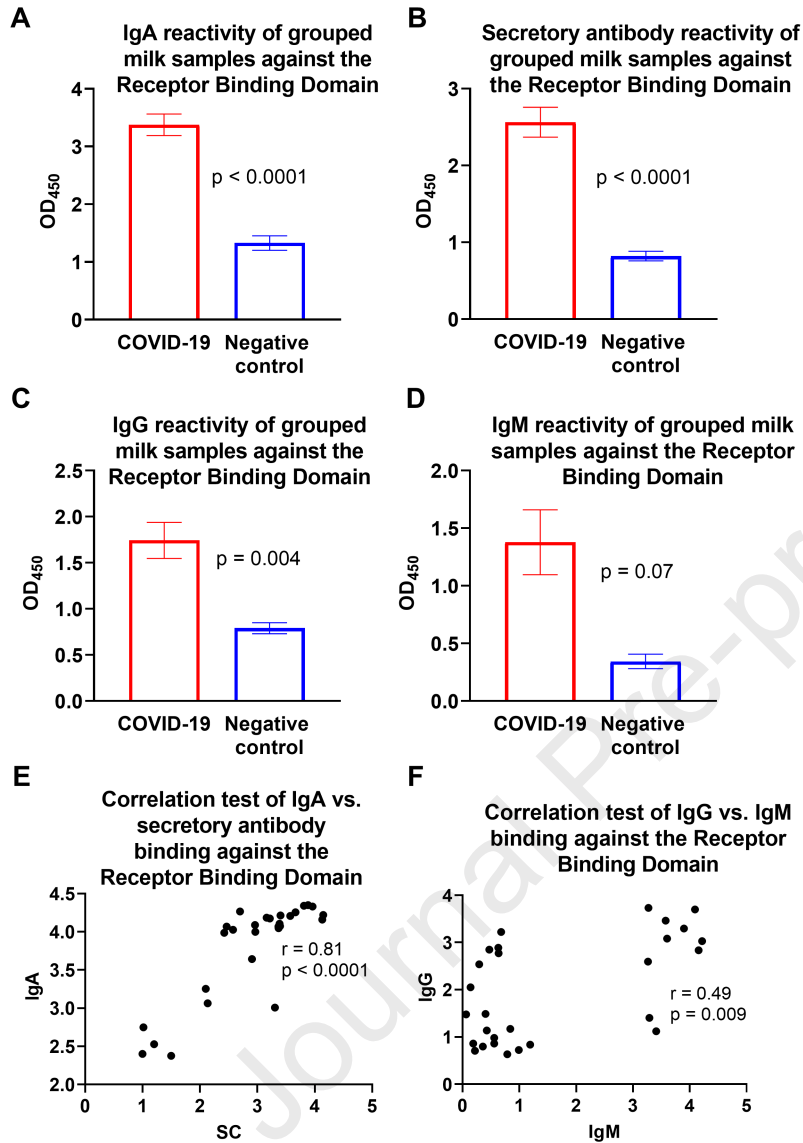
Figure 4. Milk from COVID-19-recovered donors exhibits significantly greater IgA, secretory antibody, and IgG binding against RBD compared to controls.

Highlights

- All milk from recovered donors contained significant SARS-CoV-2-specific IgA

- Most IgA could bind the Receptor Binding Domain (important neutralization epitope)

- Most Receptor Binding Domain-specific IgA was in secretory (s) form

- sIgA is durable in the mucosa, and thus potentially as a respiratory therapeutic